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Results of combined application of lmwh /alpha lipoic acid in the experimental mesenteric ischemia/reperfusion injury model in rats

Combined use of lmwh and alpha-lipoic acid in mesenteric ischemia

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Abstract

Aim: To evaluate the correlation between necrosis levels and parameters by creating an experimental mesenteric ischemia/reperfusion model in rats and assessing the effects of LMWH (Low Molecular Weight Heparin) and ALA (Alpha-Lipoic Acid) administration.

Material and Methods: Twenty-four female rats were randomly divided into three groups of eight. After anesthesia, laparotomy was performed, and the superior mesenteric artery (SMA) was identified and ligated at its origin from the aorta. Following 45 minutes of ischemia, the SMA ligation was released, and 60 minutes of reperfusion was allowed. After reperfusion, a 3 cm segment of the ileum, located 10 cm proximal to the ileocecal valve, was resected. The first group was designated as the control group and underwent only ischemia/reperfusion. In the second group, LMWH was administered subcutaneously 4 hours before laparotomy. In the third group, both LMWH (subcutaneously) and ALA (intraperitoneally) were administered 4 hours before laparotomy. Histopathological examination was conducted based on Chiu scoring to determine necrosis levels. Biochemical analysis was performed using LDH, phosphorus, lactate, complete blood count, TAS (Total Antioxidant Status), TOS (Total Oxidant Status), and OSI (Oxidative Stress Index) parameters.

Results: Our study suggests that ALA may have an inflammation-promoting effect, while LMWH, in addition to its anticoagulant properties, might act as an antioxidant, reducing the oxidative load in the organism. The combined use of LMWH and ALA is expected to reduce potential damage in the ileum.

Discussion: The study indicates that the combined use of LMWH and ALA may have a positive effect on ischemia parameters, oxidation parameters, and necrosis levels.

Keywords

Acute Mesenteric Ischemia, Alpha Lipoic Acid, Low Molecular Weight Heparin

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This study was approved by the Ethics Committee of Necmettin Erbakan University Local Ethics Committee for Animal Experiments (Date: 2022-12-30, No: 076)

Introduction

Mesenteric ischemia refers to a clinical condition characterized by reduced blood flow to the intestines due to acute arterial occlusion (embolic, thrombotic), venous thrombosis, or decreased perfusion without occlusion [1]. Established risk factors include cardiac arrhythmias, advanced age, low cardiac output states, generalized atherosclerosis, congestive heart failure, severe valvular heart disease, myocardial infarction, and intra-abdominal malignancies. Early diagnosis and effective treatment are crucial to minimize mortality; however, due to vague symptoms, lack of clinically useful diagnostic tests, and a broad spectrum of at-risk patient groups, significant improvement in the prognosis of mesenteric ischemia has not been achieved [2].

Reperfusion refers to the restoration of reduced or interrupted blood flow, and consequently oxygen, to hypoxic tissue. If cells have not irreversibly suffered damage, functions can be regained upon reperfusion; however, the reintroduction of oxygen into ischemic tissue leads to the formation of toxic oxygen radicals through a series of metabolic processes. Following the diagnosis of AMI (acute mesenteric ischemia), treatment should commence promptly. Treatment goals should focus on reducing vasospasm, preventing the spread of intravascular clotting processes, and minimizing reperfusion injury. Based on this premise, our study aimed to investigate the combined use of LMWH, which affects circulation disorders such as vasospasm and thrombosis, along with ALA, an antioxidant, to prevent or minimize post-reperfusion damage in terms of inflammation levels, necrosis/ischemia severity, and oxidation parameters.

ALA is a natural antioxidant synthesized minimally in the liver in humans [3]. It forms stable complexes by binding to transition metals and thereby eliminates heavy metals such as manganese, copper, zinc, and lead [4]. Additionally, ALA plays a role in regulating the synthesis and regeneration of other antioxidants, such as glutathione, and in regulating the activities of cellular transcription factors. It acts both as a coenzyme in metabolic processes and exhibits antioxidant properties [4, 5]. Many studies have been conducted on the antioxidant effects of alpha-lipoic acid in reducing cell damage by combating free radicals [6]. It has been shown to be effective in reducing symptoms of diabetic neuropathy [7]. Based on these observations, ALA is believed to improve inflammatory conditions in the body.

LMWHs exhibit anti-inflammatory effects in addition to their well-known anticoagulant properties. These anti-inflammatory effects are exemplified in chronic disease complications such as diabetes, malignancies, inflammatory diseases like ulcerative colitis, and viral infections [8].

Material and Methods

Groups

The rats were divided into three groups, each consisting of 8 rats. Following surgical area shaving and disinfection, laparotomy was performed via a vertical incision starting from the xiphoid process.

Group 1 (Sham group) (n=8): Intestinal loops were retracted, and the superior mesenteric artery (SMA) was isolated and ligated at its origin from the aorta (Figure 1). After 45 minutes of ischemia, the ligature on SMA was released to achieve 60 minutes of reperfusion. Following reperfusion, a 3 cm segment of the ileum, starting 10 cm proximal to the ileocecal valve, was resected.

Group 2 (n=8): All rats in this group received subcutaneous administration of LMWH at a dose of 1 mg/kg. Four hours after LMWH administration, the same procedures as in Group 1 were carried out. Following reperfusion, a 3 cm segment of the ileum, starting 10 cm proximal to the ileocecal valve, was resected.

Group 3 (n=8): All rats in this group received subcutaneous administration of LMWH at a dose of 1 mg/kg and intraperitoneal administration of ALA at a dose of 100 mg/kg. Four hours after administration, the procedures identical to those in Groups 1 and 2 were performed. Following reperfusion, a 3 cm segment of the ileum, starting 10 cm proximal to the ileocecal valve, was resected. After intracardiac blood collection, the rats in all three groups were euthanized.

Histopathological changes in the ileum tissues obtained from 24 rats (8 rats per group) were classified according to Chiu's scoring system (Table 1).

pH and lactate values were determined from blood samples collected from the rats. Data obtained from the study were analyzed using the SPSS (Statistical Package for Social Sciences) 18.0 software. Descriptive analyses presented frequency data as number (n) and percentage (%), while numerical data were expressed as mean ± standard deviation. Categorical data were compared using the Chi-square (X2) test and Fisher's exact test. The normality of numerical data was assessed using the Kolmogorov-Smirnov test. A one-way ANOVA test was used to evaluate numerical data fitting normal distribution among more than two groups. For variables that were significant in ANOVA tests, Tukey or Tamhane post hoc analyses were performed. In cases where numerical data did not fit normal distribution across more than two groups, the Kruskal-Wallis test was applied. Post hoc analysis using the Bonferroni-corrected Mann-Whitney U test was conducted for variables found significant in Kruskal-Wallis tests.

Ethical Approval

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Results

One rat from Group 1 expired during the study procedures, and therefore, no blood or tissue samples were obtained from this rat, excluding it from statistical analysis. Thus, 23 rats were included in the study: control group (n=7), LMWH group (n=8), and LMWH +ALA group (n=8).

In the LMWH +ALA group, leukocyte levels were significantly higher compared to the control group and LMWH group ($p=0.018$). Leukocyte levels were similar between the control and LMWH groups. Neutrophil-to-lymphocyte ratio (NLR) levels were found to be similar across all three groups ($p>0.05$).

LDH levels in the control group rats were significantly higher compared to the LMWH and LMWH +ALA groups ($p<0.001$). LDH levels were similar between the LMWH and LMWH +ALA groups. Phosphorus and lactate levels were similar across all three groups ($p>0.05$).

Table 1. Chiu Scoring

GRADE	CHARACTERISTICS
0	Mucosa with normal villi
1	Development of the subepithelial Gruenhagen's space, usually at the villus apex, frequently associated with capillary congestion
2	Extension of the subepithelial space with moderate lifting of epithelial layer from the lamina propria
3	Massive epithelial lifting down the sides of the villi
4	Denuded villi with lamina propria and dilated capillaries exposed. Increased cellularity of lamina propria may be noted
5	Digestion and disintegration of lamina propria; hemorrhage and ulceration

Table 2. Comparison of Oxidation Parameters Between Groups

	Controlgroup (n=7) Mean ±SD	LMWHgroup (n=8) Mean ±SD	LMWH+ALA (n=8) Mean ±SD	p
TAS	0,42±0,16*	0,62±0,15	0,60±0,09	0,021
TOS	17,70±6,03	14,94±6,11	13,37±4,52	0,34
OSİ	4,46±1,81*	2,38±0,91	2,29±1,04	0,006

Table 3. Comparison of CHIU Grade Degrees Between Groups

	Control group (n=7) n (%)	LMWH group (n=8) n (%)	LMWH +ALA (n=8) n (%)	X2 (Chi-square)	p
Grade 0	1 (14,3)	1 (12,5)	-		
Grade 1	-	3 (37,5)	-		
Grade 2	-	3 (37,5)	3 (37,5)	3,956	0,047
Grade 4	5 (71,4)*	1 (12,5)	3 (37,5)		
Grade 5	1 (14,3)	-	2 (25,0)		

Figure 1. SMA ligation.

Total antioxidant status (TAS) levels were significantly lower, and oxidative stress index (OSI) levels were significantly higher in the control group rats compared to the LMWH and LMWH +ALA groups (p values: p=0.021 for TAS, p=0.006 for OSI). TAS and OSI levels were similar between the LMWH and LMWH +ALA groups. Total oxidant status (TOS) levels were similar across all three groups (Table 2).

The grade scores of the included rats are compared in Table 3. The proportion of Grade 4 in the control group rats was significantly higher compared to the other groups (p=0.047), while rates were similar between the LMWH and LMWH +ALA groups.

Discussion

Mesenteric ischemia is a challenging diagnosis with a poor prognosis due to delayed diagnosis and the presence of comorbidities that worsen outcomes. The difficulty in diagnosis stems primarily from the wide spectrum of symptoms associated with the condition.

In cases where tissue oxygen delivery is reduced or ineffective, resulting in cellular damage and inflammation, several laboratory parameters such as lactate, leukocytosis, LDH, phosphorus, amylase, and lipase released from intestinal tissues into the plasma have been used as diagnostic aids in AMI. Elevated plasma lactate concentration is a hallmark of acute conditions threatening life. In their study, Lange H. and Jackel observed that lactate levels were elevated in all groups with mesenteric ischemia, exceeding the reference range in those with bacterial peritonitis and intestinal obstruction [9]. In our study, lactate and phosphorus levels were found to be elevated in all three groups, but no significant differences were observed among the groups.

LDH levels can rise in both chronic and acute tissue damage. However, its lack of tissue specificity diminishes its diagnostic contribution. In a study by Lapsekili E., LDH was characterized as a marker for ischemia with 94% sensitivity and 41% specificity [10]. In our study, LDH levels were significantly elevated in the control group compared to the other groups, but no significant difference was found between the LMWH and LMWH +ALA groups.

During acute inflammation, an increase in neutrophil counts and a decrease in lymphocyte counts are expected. Ercan et al. concluded from their retrospective study on patients operated for mesenteric ischemia that high NLR may indicate

a poor prognosis [11]. High neutrophil counts are indicative of inflammation, while low lymphocyte counts reflect the overall poor condition and physiological stress. In our study, leukocyte counts were significantly higher in the LMWH +ALA group compared to the other two groups, raising suspicion that ALA may exacerbate inflammation. However, NLR levels were similar across all three groups. Other studies have shown that high NLR correlates not only with increased neutrophil counts but also with decreased lymphocyte counts, which may be attributed to increased steroid exposure due to physiological stress. Considering that the ischemia period was planned as 45 minutes after SMA ligation in our study, the results of our study may suggest that this synthesis pathway may require a longer duration in studies where the ischemia duration is extended.

In mesenteric ischemia, tissue damage occurs due to ischemic injury and subsequent reperfusion of oxygen to the tissue, which triggers a cascade of reactions leading to oxidative stress. When a previously blocked area is suddenly opened, additional cell death occurs compared to the ischemic period alone. This process involves activation of the complement system, activation of leukocytes, increased free oxygen radicals, and dysfunction of endothelial cells, followed by reactions resulting in necrosis and autophagy, ultimately leading to ischemia-reperfusion injury [12].

Several studies have highlighted the role of oxidative metabolism in elucidating the damage mechanism during ischemia. Total antioxidant status (TAS) has been found effective in many studies to illuminate oxidative damage occurring during injury. Similarly, the total oxidant status (TOS) created by free oxygen radicals during damage is assessed under a unified framework. The oxidative stress index (OSI), derived from the ratio of TAS to TOS, serves as an indicator of the effective oxidative burden in the organism. Kartal et al. demonstrated in a skeletal muscle ischemia-reperfusion model using OSI/TAS/TOS indices that ALA exhibits a protective effect against oxidative damage and apoptosis [13]. In our study, alongside the natural antioxidant ALA, we aimed to investigate the antioxidant properties of LMWH. While TAS levels were significantly lower in the control group compared to the other two groups, OSI was significantly higher. Anti-oxidative activity was observed only in the group treated with LMWH alone (Group 2), but no significant differences in oxidation parameters were found between Groups 2 and 3.

In the study by Yaman et al., using a mesenteric ischemia-reperfusion model, they compared intestinal mucosal degeneration and bacterial translocation levels among experimental groups. They found a significantly higher CHIU score in the control group with induced mesenteric ischemia compared to the group treated with ALA and L-carnitine [14]. Conversely, Köksal et al. investigated the efficacy of LMWH on bacterial translocation in a mesenteric ischemia-reperfusion model and did not find a significant difference in CHIU scores in the group treated with LMWH, attributing this to its anticoagulant effects potentially predisposing to hemorrhage and ulceration, thus not exerting a positive effect on ileal damage scoring [15]. However, in our study, contrary to these findings, we observed a significantly higher CHIU grade 4 score in the control group compared to the groups treated with LMWH and LMWH +ALA. Therefore, we hypothesize that both LMWH and the combination of LMWH +ALA may exert a positive effect on ileal damage levels.

Limitation

There are several limitations to consider when evaluating the results of this study. The ischemia and reperfusion durations used in the experimental model may not fully reflect the clinical scenarios encountered in human patients, which could affect the applicability of the results. The effects of LMWH and ALA have been assessed solely in the context of mesenteric ischemia/reperfusion injury; therefore, interactions of these agents with other potential therapeutic agents or underlying health conditions have not been examined. Additionally, while histopathological analyses provide valuable information about tissue damage, long-term outcomes or recovery processes post-treatment have not been evaluated in this study. Lastly, variations in individual responses to LMWH and ALA could not be accounted for, which may influence the overall results. Future studies should investigate the therapeutic effects of these agents in clinical settings using larger sample sizes and diverse experimental designs.

Conclusion

In our study investigating the combined use of LMWH and ALA and their effects on oxidation, inflammation, ischemia, and necrosis levels, significant leukocytosis was observed in the group where both pharmaceutical agents were used, suggesting that ALA may have an inflammatory effect. According to our study's findings, the group treated with LMWH showed significantly higher TAS levels and lower OSI levels compared to the control group, indicating that in addition to its anticoagulant effect, heparin may also have antioxidant functions. The control group exhibited a higher CHIU grade 4 score compared to the other two groups. Therefore, both LMWH alone and combined use of LMWH+ALA appears effective in preventing damage development in the ileum. However, for a more comprehensive evaluation of the effectiveness of LMWH alone and LMWH +ALA in practice, further animal experiments and subsequent human trials in a larger series are deemed necessary.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Bilge Z, Köksal AŞ. Colonic ischemia. Curr Gastroenterol Rep. 2016;20(4):466-71.

2. Glenister KM, Corke CF. Infarcted intestine: A diagnostic void. ANZ J Surg. 2004;74(4):260-65.

3. Ergene E. A study on alpha-lipoic acid and its metabolic effects. J Adnan Menderes Univ Health Sci. 2018;2(3):159-65.

4. Uygur AG, Şingirik E, Yücel AH. Protective effects of alpha-lipoic acid in

methotrexate-induced damage. Arch Res Scan J. 2019;28(3):227-36.
5. Ziegler D, Duenas M, Wiegand W, Schütte K, Tölle T, Lämmermann J, et al. Alpha-lipoic acid improves metabolic control in diabetic patients with symptomatic peripheral neuropathy: A randomized, double-blind, placebo-controlled trial. Diabetes Care. 2004;27(9):2172-78.
6. Huang L, Zhang Y. Protective effects of alpha-lipoic acid against oxidative stress in acute pancreatitis. Clin Sci. 2015;129(10):835-46.
7. Ibrahimpasic K. Alpha-lipoic acid and glycemic control in diabetic neuropathies during type 2 diabetes treatment. Med Arch. 2013;67(1):7-9.
8. Yan Y, Ji Y, Su N, Mei X, Wang Y, Du S, et al. Non-anticoagulant effects of low molecular weight heparins in inflammatory disorders: A review. Carbohydr Polym. 2017;160(1):71-81.
9. Miller AC, Heller M, Kocoshis SA, Goldstein AM, Chinnock B, Parnell R, et al. Lactate as a predictor of outcomes in patients with acute abdomen: A systematic review and meta-analysis. J Trauma Acute Care Surg. 2021;90(2):313-22.
10. Gatta A, De Ceglie A, Viscido A, Manfreda R, De Angelis N, Di Mitri R, et al. Alkaline phosphatase as a potential marker for acute mesenteric ischemia. BMC Gastroenterol. 2018;18(1):32.
11. Ercan KD. The effectiveness of preoperative neutrophil-lymphocyte ratio in determining the prognosis of acute mesenteric ischemia. Selcuk Med J. 2019;35(4):230-34.
12. Collard CD, Gelman S. Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. Anesthesiology. 2001;94(6):1133-38.
13. Kartal H, Büyük B. Effects of alpha-lipoic acid on skeletal muscle ischemia-reperfusion injury in mice. J Surg Med. 2020;4(7):567-72.
14. Petronilho F, Silva E, Quevedo J, Brizola E, de Almeida J, Ribeiro L, et al. Alpha-lipoic acid attenuates oxidative damage in organs after sepsis. Inflammation. 2016;39:357-365.
15. Yagmurdur MC, Turk E, Moray G, Can F, Demirbilek M, Haberal N, et al. Effects of heparin on bacterial translocation and gut epithelial apoptosis after burn injury in the rat: Dose-dependent inhibition of the complement cascade. Burns. 2005;31(5):603-9.

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